Patellae were extracted from New Zealand White rabbits immediately after sacrifice. Tissue samples were incubated in ECM-marking dye for 5 hours prior to testing. Static compressive loads were applied to the samples in this order: 10%, 20%, 30%, 40%, 60%, and 80% strain. Confocal image sections were recorded before and at each load after 15 minutes. Local ECM strain and cell deformations were analyzed to quantify the overall response of the cartilage and cells.
Local compressive ECM strains increased with increasing applied nominal tissue deformation, from 20.4% to 58.6% at 10% and 80% applied tissue strain, respectively. Chondrocyte volume increased slightly under a 10% nominal tissue strain, then decreased at a 20% strain, followed by a further decrease at 30%, before remaining essentially constant up to 80% applied tissue strain. Cell height compressive strain increased in a similar fashion to the ECM strain. Cell strains in the transverse directions (width and depth) remained approximately uniform for nominal tissue strains ranging from 10% to 60%, however at 80% nominal tissue strain, the transverse cell dimensions increased greatly.
These results provide new insight into the deformation behavior of chondrocytes in their native environment under physiological and extreme nominal tissue strains. The relative stability of cell volumes and the transverse dimensions of cells under increasing tissue loads is consistent with the hypothesis that the extra- and peri-cellular matrices protect cells from excessive strains in situations of large cartilage deformation.

973-Pos Board B759
Evaluation of Quantitative Approaches for High Precision 3D Single Particle Tracking in Thick Cellular Specimens using Multifocal Plane Microscopy
Ramraj Velmurugan[1], Sripad Ram[1], Anish V. Abraham[2], Jerry Chao[2], Andrea Grosso[2], E. Sally Ward[1], Raimund J. Ober[2].
[1]UT Southwestern Medical Center, Dallas, Dallas, TX, USA, [2]University of Texas at Dallas, Dallas, TX, USA.
Single particle tracking in live biological samples has been limited in application because of the lack of ability to follow the particles in the axial direction for significant distances. Further, imaging just one single focal plane leads to a significant decrease in axial localization accuracy when the point source is in focus. MUltifocal Microscopy (MUM) is a method that has been used to obtain high accuracy 3D localization information by simultaneously imaging more than one focal plane in the sample [1]. It has previously been used to image multiple closely-spaced focal planes to study intracellular biological events [2, 3].
We have significantly expanded on the capabilities of MUM wherein we image at multiple well-separated focal planes in the sample to track single particles across depths of several microns. Using more than two planes allows us to monitor very thick samples and at the same time further increase the accuracy of axial localization near the focal planes. The widefield imaging configuration of MUM in conjunction with large plane-spacings allows us to simultaneously track multiple single particles for extended periods of time in three dimensions.
We evaluate quantitative methods of estimating the axial localization of point sources using data from multiple focal planes and compare various methods of estimation to the lower bounds of axial localization accuracy. We then show that such quantitative imaging can be used to simultaneously track multiple quantum dots across the axial length of live cells with high spatial and temporal resolution for extended periods of time. Such ability opens up new possibilities in quantitative single particle tracking applications in biology.
1. IEEE Trans. Nanobioscience, 2004, 3:237-242.
2. PNAS, 2007, 104:5889-5894.
3. Biophys J., 2008, 95:6025-6043.

974-Pos Board B760
Single-Cell Raman Spectroscopy and Morphological Changes of Normal and Cancer Cells in Suspension
Silki Arora, Sang Hoon Park, Lisa Ritchey, Richard Ottman, Ratna Chakrabarti, Alfons Schulte.
University of Central Florida, Orlando, FL, USA.
Spatially resolved spectroscopies provide a non-destructive in-situ probe at the single cell level. We employ micro-Raman spectroscopy to investigate the biochemical and structural composition of P69 and PC3 prostate cells. P69 is a non-tumorigenic cell line developed from normal prostate epithelial cells while PC3 is a highly aggressive metastatic prostate cancer cell line developed from bone metatasis. The studies were conducted to differentiate between benign and malignant cancer tissues. Micro-Raman spectra were measured with 633 nm excitation with less than 4 mW power on live cells both in suspension and on substrates. The Raman spectra reveal differences between normal and cancer cells in the C-C, amide I and amide III regions. We also investigate mechanical properties and morphological changes in individual cells employing high pressure microscopy. Our setup allows real time imaging of individual cells at variable pressure. We probe differences in cell mobility and mechanical stability between normal and cancer cells during a pressure cycle over the range from 0.1 to 200 MPa.

975-Pos Board B761
High Content Study of Vesicular Trafficking in Polarized Cells
Javier Mazzaferri[1], Stephane Lefrançois[1,2], Santiago Costantino[1,3].
[1]Centre de Recherche de l'Hôpital Maisonneuve-Rosemont, Montréal, QC, Canada, [2]Département de Médecine, Université de Montréal, Montréal, QC, Canada, [3]Institut de Génie Biomédicale, Université de Montréal, Montréal, QC, Canada.
The molecular mechanisms involved in the intracellular trafficking of proteins that sustain cell polarization in epithelial cells has not been fully elucidated. Traditionally, the evolution of protein localization has been studied by assessing protein localization on a time-lapse basis. This kind of approach is unable to reveal the fast-occurring details of vesicular transport that could be key to understanding certain aspects of the molecular machinery. Additionally, this problem is often addressed qualitatively or with a rather low number of observations yielding results that could be biased and not statistically significant. We propose to use in-vivo imaging and a battery of image processing tools to study apical vs. basolateral trafficking with a high-content approach.
Aquaporins (AQP) are a family of water-channel proteins present on specific membrane domains of mammalian cells. For example, it is known that AQP4 is localized to the basolateral membrane, while AQP2 is localized to the apical membrane. This differential localization makes these proteins a proper model for studying apical vs. basolateral protein sorting. We have in-vivo imaged MDCK cells transfected with GFP-AQPs at near 10 Hz frame rate with a conventional epi-fluorescence microscope, and the resulting movies processed using a single particle tracking algorithm to obtain the trajectories of the vesicles. The significant number of detected tracks (sometimes greater than 1000 per cell) is further analyzed by assessing the statistical distribution of several descriptors, for example, vesicle mean speed, direction of movement, effective protein transport and motion type. The results obtained in a population of wild-type MDCK cells will be presented and discussed.

976-Pos Board B762
Three-Dimensional Structural Dynamics in Contracting Myocytes of Beating Chick Embryo Hearts Imaged with Nonlinear Microscopy
Masood Smim, Ian Tretyakov, Nicole Prent, Virginijus Barzda.
University of Toronto, Mississauga, ON, Canada.
Capturing high-speed structural dynamics of contracting myocytes is an important requirement for understanding the biophysical processes occurring during contraction. We present our recent technological innovations that allow for real-time three-dimensional (3D) observation of the moving cardiac muscle tissue in beating chick embryo hearts. In vivo dynamics in the beating heart muscle tissue are visualized using the multicontrast nonlinear optical microscope. The setup employs two femtosecond laser beams with staggered pulse trains, which allow for the simultaneous measurement of second harmonic generation at perpendicular laser polarizations. Fast refocusing deep inside the sample is achieved by the deformable mirrors, which allow imaging moving structures in 3D. The microscopy setup revealed intricate interactions between sarcomeres during contracting and quiescent periods of the myocytes. The high-speed 3D imaging capabilities provide exciting opportunities to investigate fundamental biophysical mechanisms that underlie various fast biological processes occurring in a 3D volume in living organisms.

977-Pos Board B763
Neutrophil Sling: a New Cell-Autonomous Adhesive Structure Enabling Rolling at High Shear Stress
Prithu Sundd[1], Edgar Gutierrez[2], Maria K. Pospieszalska[1], Ekaterina Koltsova[1], Yoshihiro Kuwano[1,3], Satoru Fukuda[3], Alexander Groisman[2], Klaus Ley[1].
[1]La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA, [2]University of California San Diego, La Jolla, CA, USA, [3]University of Tokyo, Tokyo, Japan.
Neutrophil rolling is mediated by P-selectin on the endothelium binding to P-selectin-glycoprotein-ligand (PSGL)-1constitutively expressed on neutrophils. In post capillary venules *in vivo*, neutrophils roll at shear stresses of 2-35 dyn/cm^2. Recently, quantitative dynamic footprinting (qDF) microscopy